ELECTRON MICROSCOPE OBSERVATIONS OF THE TRICHOCYSTS AND CILIA OF PARAMECIUM

M. A. JAKUS AND C. E. HALL

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

In previous publications, electron micrographs have been shown of trichocysts (Jakus, 1945) and of cilia (Schmitt, Hall, and Jakus, 1943). Recently we have re-examined both these organelles using the shadow-casting technique of Williams and Wyckoff (1945). The new technique shows structural detail with improved clarity and reveals some features not previously visible in specimens prepared in the conventional manner.

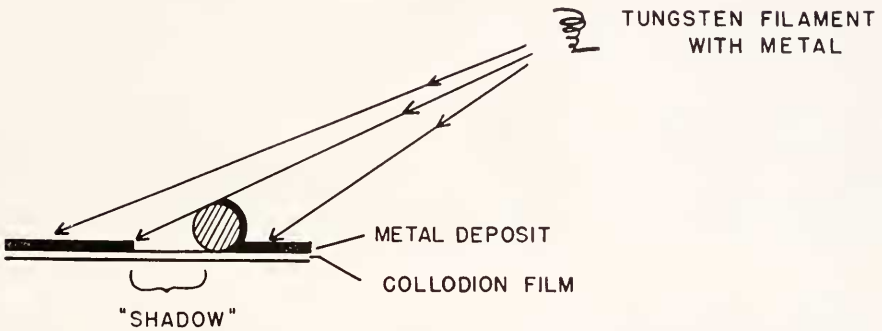


FIGURE 1. Diagram of shadow-casting technique.

The shadow-casting technique is illustrated diagrammatically in Figure 1. A specimen is placed in a vacuum bell-jar containing a conical tungsten filament in which are placed some small pieces of a suitable metal such as chromium. When the filament is raised to a high temperature by the passage of an electric current the metal evaporates, travelling in straight lines and depositing on the specimen as indicated. Structures projecting above the surface of the supporting film cast permanent shadows to the "leeward" and intercept metal to the "windward." Specimens are then examined in the electron microscope in the usual manner. In positive prints the shadows appear bright because they represent relatively transparent regions in the object. It is customary, therefore, to prepare micrographs as negative prints so that the shadows will appear darker than the background.

TRICHOCYSTS

The structure and properties of the trichocysts of Paramecium have been described in a previous paper (Jakus, 1945). In electron micrographs, the discharged trichocyst consists of a sharply-pointed tip and an elongated, cross-striated shaft with a periodicity of about 550 A. The cross-striated structure appears to be a

thin membrane formed by the lateral aggregation of fine fibrils. The tip, in contrast to the shaft, is quite opaque. The reason for this opacity was not obvious.

Further information about the morphology of the dried extruded trichocyst is obtained from electron micrographs of shadowed specimens (Fig. 2). The tip is seen to be a compact structure which stands up from the film and is not flattened to any great extent as a result of dehydration. The contour of its shadow indicates that it is shaped somewhat like a golf tee. In contrast to the tip, the dried shaft is very flat, as is evident from the short shadow it casts. The cross striations previously observed are enhanced by the metal, indicating that the surface has a regularly corrugated contour. The elevated regions correspond to the darker bands in both untreated trichocysts and those stained with phosphotungstic acid. Other details of structure observed previously may also be found in some shadowed trichocysts. These are the fine longitudinal striations of the shaft membrane and the larger periodicity (2,200 A) frequently noted along the shaft. The latter may appear simply as a slight further intensification of every fourth dark band, suggesting that these ridges have a somewhat higher elevation than do the others.

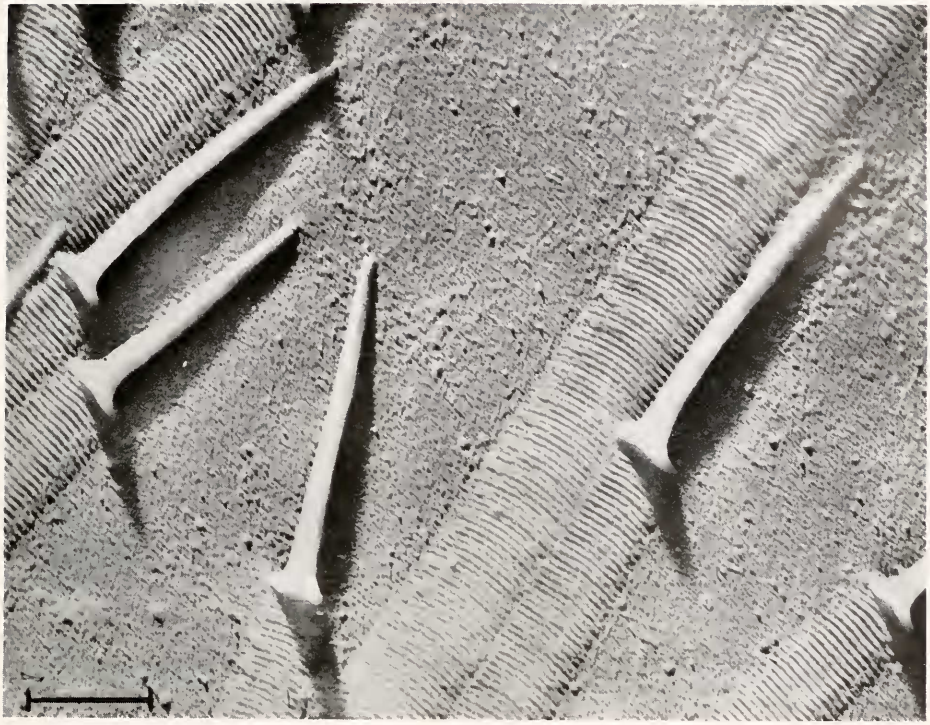
In some specimens the pointed tip appears regularly cross-striated, if the amount of metal deposited has not been excessive and the orientation of the tip is approximately parallel to the direction of deposition. This banding has not been seen in either stained or unstained specimens and, while it is readily visible in the original micrographs of shadowed tips, it is not considered to be of sufficient clarity for reproduction. Although relatively constant in any one tip, the spacing varied from 280 to 365 A in the different tips measured and had an average value of about 300 A. This is to be compared with the average period of about 550 A in the trichocyst shaft.

Cilia

The cilia of Paramecium are shed quite readily if the cell is injured and both intact cilia and fragments are observed frequently in preparations of trichocysts. Each cilium consists of a bundle of fibrils (about eleven in number), extending the full length of the cilium (Fig. 3). The diameter of the dried fibrils lies between 300 and 500 A. It may be of significance that both the number of fibrils and their diameter are in close agreement with the corresponding values observed in the sperm tails of numerous animal forms (Schmitt, Hall, and Jakus, 1943).

In fixed preparations (for example, with OsO_4), the component fibrils usually adhere to form a compact bundle, while in unfixed cilia they separate to a greater or lesser extent. They are clearly defined in shadowed specimens. Usually the separation of fibrils is not complete and they remain in close contact near the end of the cilium which was attached to the cell. Here they appear sometimes to be joined into two closely adjacent bundles.

It is not evident what holds the fibrils together in the living cilium. No spiral sheath similar to that observed in mammalian sperm tails (Schmitt, Hall, and Jakus, 1943) or in Euglena flagella (Brown, 1945) has been seen. If a sheath does exist, it must be very fragile and easily ruptured. In some cilia, a rather poorly-defined cross-striation has been noted, particularly in two or more adjacent fibrils. This striation appears to be unlike that of clearly cross-striated proteins and, if it is not an inherent periodicity in the fibril, it may represent the remnants of some binding or enveloping structure.



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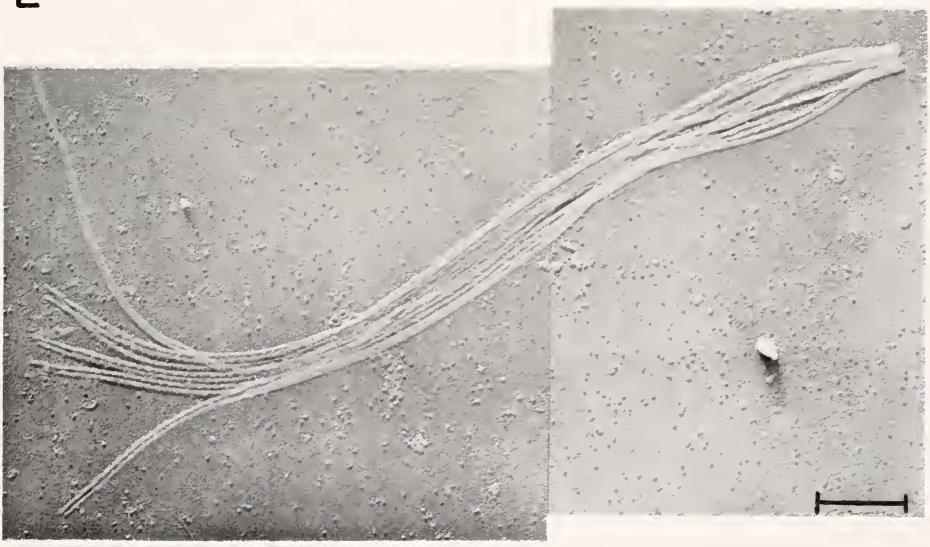

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FIGURE 2. Trichocysts from Paramecium, shadow-cast with chromium. × 16,000.
FIGURE 3. Cilium from Paramecium, shadow-cast with chromium. × 11,000.

Summary

Electron micrographs of shadow-cast trichocysts of Paramecium show that the dried trichocyst shaft is flattened on the supporting film, while the pointed tip is apparently more resistant to collapse on dehydration. Accentuation, by the metal, of the cross striation previously observed in the shaft indicates that the periodicity is accompanied by corrugation of the dried surface. A cross striation in the tip is also visible in some micrographs of shadow-cast specimens. In the few cases where the periodicity could be measured, the average spacing was about 300 A, as compared to about 550 A for the well-defined shaft striation.

In electron micrographs of shadow-cast specimens of Paramecium cilia, the component fibrils are seen with greatly increased clarity.

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